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Stable gene transfer and tissue-specific expression of a human globin gene using adenoviral vectors

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Helper-free double recombinant adenoviruses containing a genomic human globin gene and the neomycin resistance gene (*neo*^{R}) have been constructed. The inserted globin and *neo*^{R} genes are stable and transcription of two human globin genes (β and a hybrid $\gamma-\beta$ gene) is correctly initiated at the respective globin promoter during lytic infection in 293 cells. The *neo*^{R} gene driven by the SV40 early promoter confers G418 resistance to human fibroblasts and K562 human erythroleukemia cells transformed with these viruses. Most *neo*^{R} clones contain the entire recombinant viral genome, including the inserted globin gene, integrated into their chromosomes. Normally, K562 cells express their γ but not their β globin genes. The transferred human β globin gene was not expressed in either K562 cells or fibroblasts. However, the hybrid $\gamma-\beta$ globin gene was expressed in all K562 clones that contained the gene whereas $\gamma-\beta$ mRNA was barely detectable in the fibroblasts. This demonstrates tissue-specific expression of the adenovirus-transferred globin gene. Furthermore, the two transferred genes, globin and *neo*^{R}, which are situated more than 20 kb apart in the viral genome appear to be independently regulated.

Key words: globin gene regulation/K562 cells/adenoviral vectors

Introduction

Recombinant adenoviruses have been used successfully to transfer genes into cells to obtain transient expression of the transferred gene. Proteins that are difficult to purify due to low abundance have been introduced into cell lines using recombinant adenoviruses to obtain high level protein expression. The SV40 large T antigen (Thummel *et al.*, 1981, 1982, 1983) and the polyoma virus tumor antigens (Mansour *et al.*, 1985) have been produced in this fashion. Genes encoding enzymes (Berkner and Sharp, 1984; Yamada *et al.* 1985), rat albumin (Babiss *et al.*, 1986) and the mouse immunoglobulin μ gene (Ruether *et al.*, 1986) have also been transferred into cells and expressed without integration into the cellular chromosomes.

We and others have used adenoviruses to integrate stably selectable marker genes into chromosomes of tissue culture cells. Adenovirus vector systems lacking either E1A and E1B transforming genes or E1A only have been developed and used to propagate different selectable marker genes: neomycin resistance (*neo*^{R}) gene, SV40 T antigen, dihydrofolate reductase (DHFR) gene and thymidine kinase (*tk*) gene. These recombinant viruses were used to transform a variety of cell types and to express selectable genes (Van Doren *et al.*, 1984; Van Doren and Gluzman, 1984; Berkner and Sharp, 1984; Haj-Ahmad and Graham, 1986) including hematopoietic cell lines (Karlsson *et al.*, 1985). Since the *neo*^{R}-containing adenoviral vector could transform a variety of cell lines we evaluated the ability of this vector system to transfer and express a non-selectable gene after integration of the recombinant adenovirus vector into the chromosomes of the recipient cells.

The adenovirus vector system has many advantages. First, the large 33-kb adenoviral recipient vectors can accommodate two genes located more than 20 kb apart in the E1 and E3 regions of the viral genome (Gluzman *et al.*, 1982). This should favor independent regulation of the two genes. Second, the E1A and E1B transforming genes have been removed from the vector which prevents normal adenoviral transformation of infected cells and keeps the various promoters along the viral genome relatively inactive. Third, these adenoviral vectors can be easily propagated in E1 producing 293 cells to produce helper-free recombinant viruses to high titers (10^9 p.f.u./ml) in contrast to SV40 recombinant virions that cannot be propagated to a high enough titer (10^6-10^7 p.f.u./ml) using the COS cell helper system (Gluzman, 1981) and therefore require the presence of a helper virus (Karlsson *et al.*, 1985). Fourth, since adenoviruses are DNA viruses, difficulties with splicing of introns from inserted genomic genes do not occur as in retroviral vectors (Shimotohno and Temin, 1982). Fifth, no difficulties should be expected from antisense RNA preventing adequate protein expression as is seen in retroviral vectors carrying genomic genes in the opposite orientation of viral transcription (Cone *et al.*, 1986; S.Karlsson, S.Schweiger, Th.Papayannopoulou, G.Stamatoyannopoulos and A.W.Nienhuis, submitted).

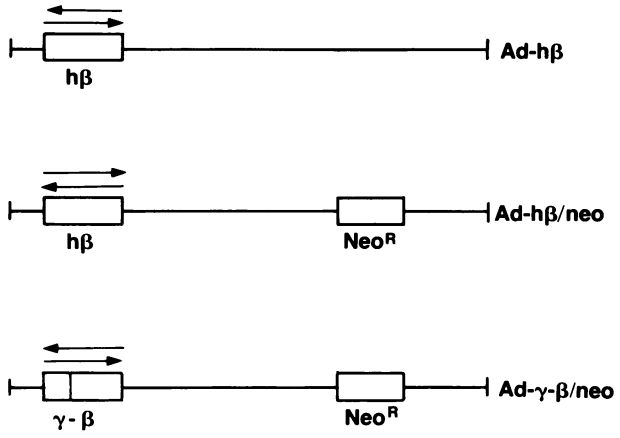

Fig. 1. Three recombinant adenoviruses containing a human genomic globin gene in the E1 region. hβ indicates the human β globin gene, $\gamma-\beta$ indicates a hybrid gene made of two human globin genes, γ (5' part) and β (3' part). *Neo*^{R} is the neomycin resistance gene situated in the E3 region. The human β globin gene insert is 2976 bp long and spans from the *Hpa*I site ~800 bp upstream from the β globin CAP site to the *Pst*I site ~500 bp downstream from the transcription termination site. The human $\gamma-\beta$ globin gene is a 2541-bp long hybrid gene starting at the *Stu*I site 386 bp upstream from the γ globin CAP site, and is joined to the β gene at the *Bam*HI site at the end of exon 2. The 3' downstream part is the same as for the human β globin gene described above.

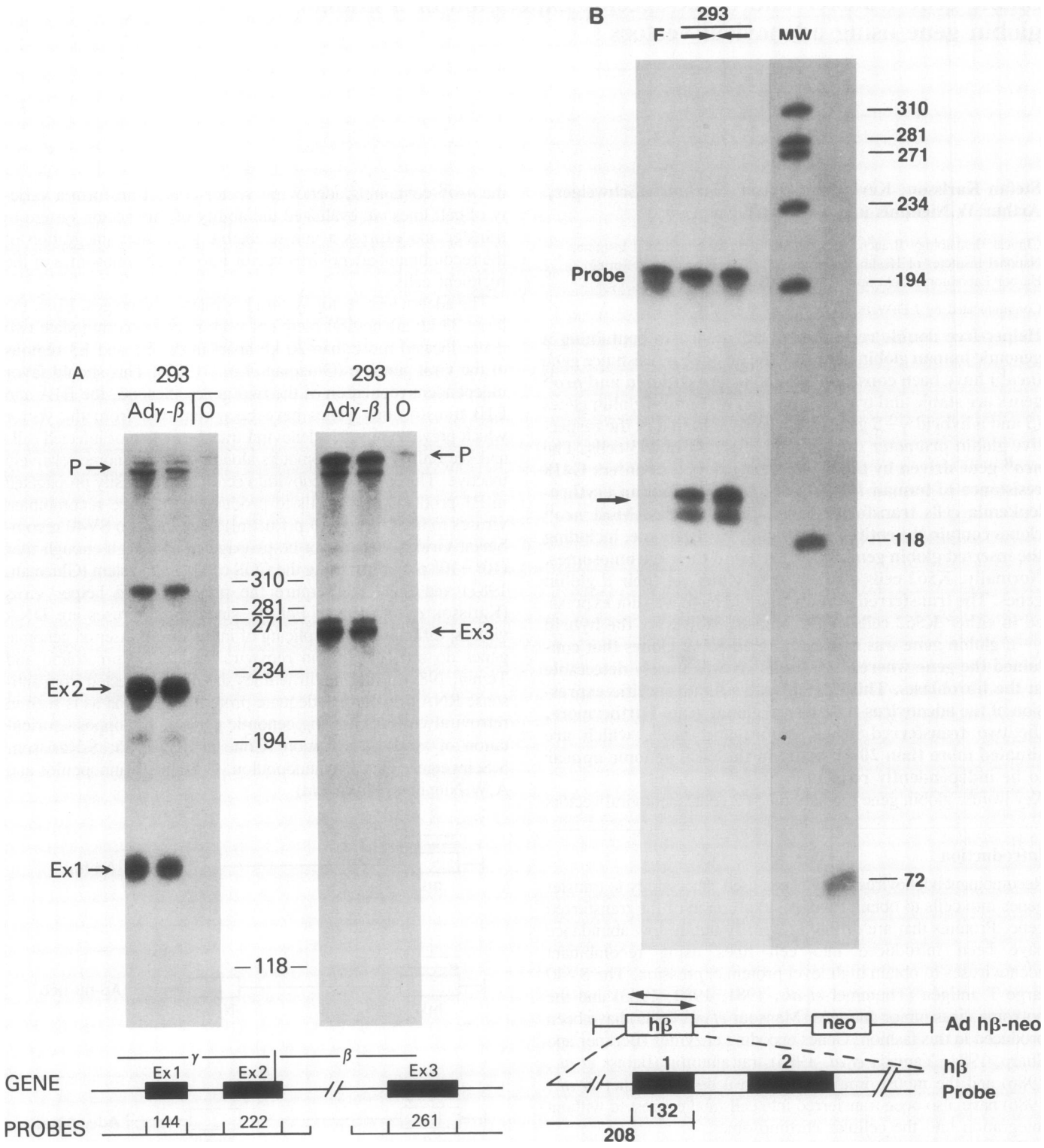

Fig. 2. Expression of globin genes during lytic infection of 293 cells by Ad-γ−β/neo and Ad-hβ/neo. RNA was isolated 28 h after infection of 293 cells and analysed by S1 nuclease mapping. **A** shows analysis of RNA from cells infected with Ad-γ−β/neo and probed with a 5′ probe detecting exon 1 (144 nucleotides) and exon 2 (222 nucleotides). On the right in (**A**) the 261 nucleotide exon 3 fragment is seen. P denotes the signal from the probe. (**B**) shows proper initiation of the human β globin gene in two Ad-hβ/neo viruses that contain the human β globin gene in the forward (→) or opposite (←) orientation.

Globin gene expression has been extensively studied during the last few years and both human and murine hematopoietic cell lines exist that express globin genes in a tissue- and stage-specific manner (reviewed by Karlsson and Nienhuis, 1985). We decided to ask whether adenoviral vectors could effectively transfer and express a genomic human globin gene in a regulated fashion. Our results show that the transferred human γ−β globin gene expresses in K562 cells but the transferred β globin gene does not. Neither gene is expressed effectively after introduction into human fibroblasts. These results are in agreement with the ex-

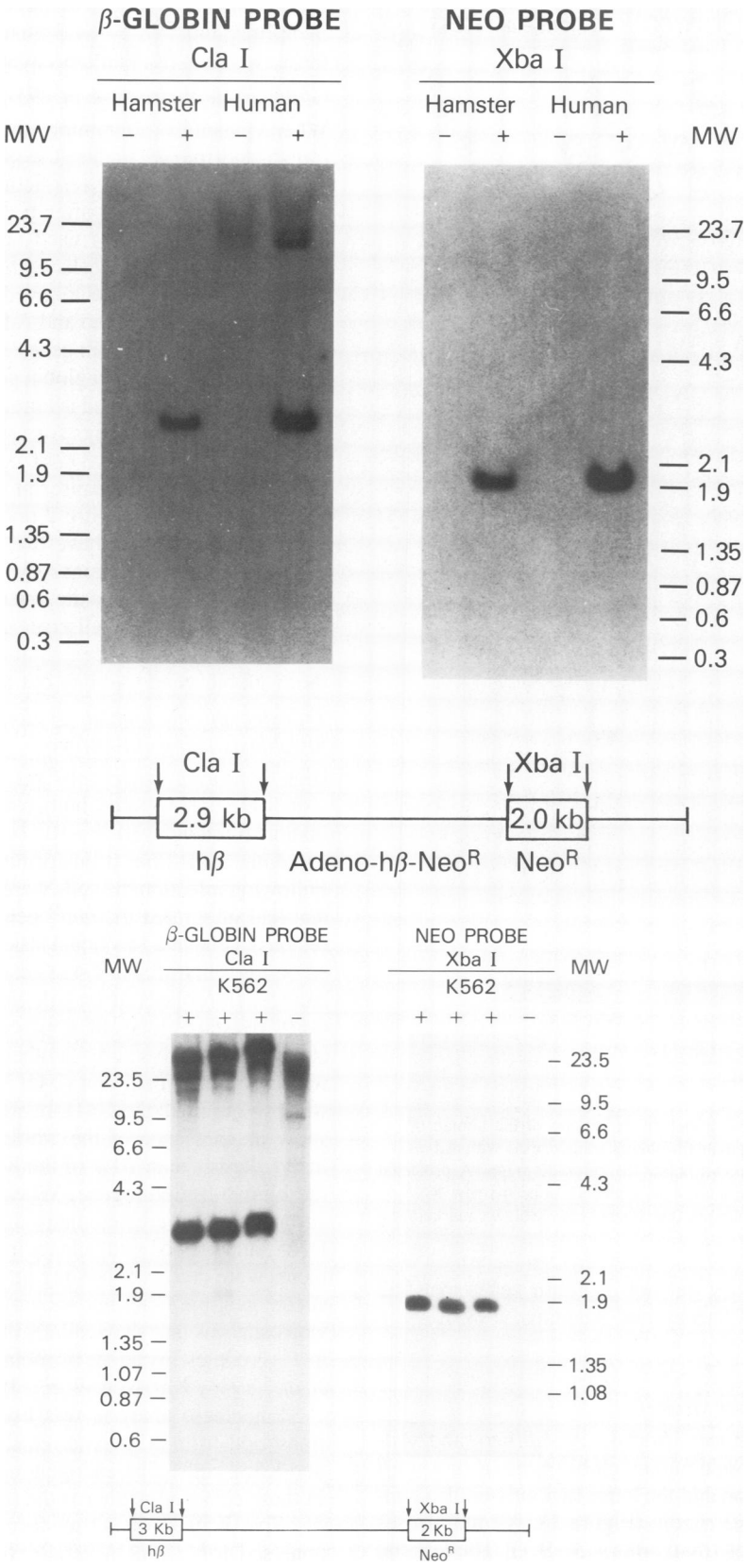

Fig. 3. Southern blot analysis showing successful transfer of human β globin and the neomycin resistance genes into K562 cells and fibroblasts after infection with Ad-hβ/neo and subsequent selection in G418. **A** demonstrates the 3-kb human globin gene in Ad-hβ/neo transformed human and hamster fibroblasts when the DNA is cut with *Cla*I and the filter probed with a human β globin probe. Similarly, these same DNA samples are shown to contain the 2-kb *neo*^{R} gene insert after cutting with *Xba*I and probing the filter with a neo probe. + indicates virally transformed cells. − indicates control cells. **B** shows the detection of the 3-kb human β globin gene and the 2-kb *neo*^{R} gene in K562 cells using the same enzymes and probes as in **A**. The endogenous human β globin gene is also seen in the human cells as a high mol. wt band since *Cla*I does not cut frequently in the human β gene cluster.

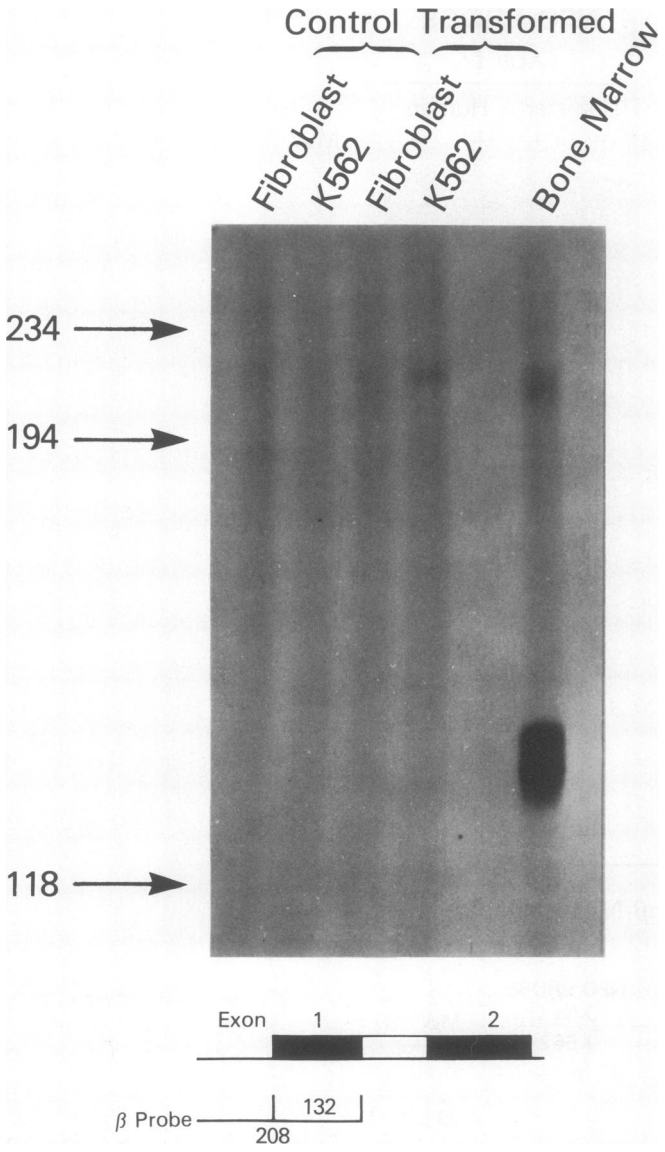

Fig. 4. S1 nuclease analysis showing lack of expression of the human β globin gene in K562 cells and human fibroblasts transformed with Ad-hβ/neo. The β globin probe is shown below the autoradiograph. A protected fragment of 132 nucleotides from exon 1 is seen with bone marrow RNA but not with RNA from K562 cells and the fibroblasts. Mol. wt markers are shown on the left.

pression of the endogenous γ but not β globin genes in K562 cells (Dean *et al.*, 1983; Charnay and Maniatis, 1983).

Results

Construction of recombinant viruses

The recombinant adenoviruses Ad-hβ, Ad-hβ/neo and Ad-γ−β/neo all contain a genomic human globin gene (β or a γ−β hybrid gene) in the E1 region and the latter two viruses also contain the *neo*^{R} gene in the E3 region (Figure 1). All the viruses lack the E1A and E1B genes (map units 1.3−9.1). The left end of the virus contains 454 nucleotides of viral sequences followed downstream by the globin gene insert (in place of the E1 genes). The 454-bp left end harbors an origin of viral DNA replication, the sequences necessary for packaging, the E1A enhancer (Hearing and Shenk, 1983) and most of the E1A promoter (except the TATA box). The viruses that contain the *neo*^{R} gene in the E3 region have a deletion of wild-type *Xba*I-D fragment (map units 78.5−84.3). The *neo*^{R} gene is inserted between these two sites (K.Van Doren and Y.Gluzman, in preparation).

Globin gene expression in 293 cells

When plasmids containing the human β globin gene are transfected into 293 cells, the globin gene is readily expressed without the presence of a *cis*-acting enhancer element as detected by RNA analysis 48 h after transfection (Green *et al.*, 1983). The adenoviral vectors used here replicate in 293 cells. We therefore infected 293 cells with our adenoviral vectors to look at the expression of the inserted globin genes. As is shown in Figure 2 both Ad-hβ/neo and Ad-γ−β/neo recombinant viruses expressed inserted globin genes 28 h after infection of 293 cells. The human γ−β hybrid globin gene RNA is initiated at the CAP site and is efficiently and accurately spliced as indicated by protection of DNA fragments corresponding to exon 1 (144 bp), exon 2 (222 bp) and exon 3 (261 bp) of the hybrid γ−β gene. The human β globin gene transcript is also properly initiated, spliced and terminated (Figure 2 and data not shown). Most of the viruses analysed here have the globin gene insert in the same orientation as the E1 genes (5′→3′ is left to right) but globin gene inserts in the opposite orientation have also been found to be functional in 293 cells (see for example Ad-hβ/neo Figure 2). Similar data have been obtained with viruses containing only the β globin gene (Ad-hβ) but not the *neo*^{R} gene (not shown).

Transfer of the human β globin gene into K562 cells and fibroblasts using Ad-hβ/neo

High titer viral lysates ($>10^8$ p.f.u./ml) containing the Ad-hβ/neo virus were used to infect human and hamster fibroblasts (transformation frequency $10^{-2}-10^{-3}$) and the K562 human erythroleukemia cell line (transformation frequency $10^{-4}-10^{-5}$). Following infection the cells were selected in G418 to obtain cells that had integrated the *neo*^{R} gene into the chromosomes of the recipient cells in a functional state. Further analysis was performed on mass cultures of G418-resistant populations of human and hamster fibroblasts or on cell cultures obtained from individual G418 resistant clones of K562 cells. DNA from these cells was isolated and analysed by Southern blot analysis (Figure 3). The whole transcriptional unit (2-kb *Xba*I fragment) of the *neo*^{R} gene was present in all the G418-resistant cells. The same DNAs also show the presence of the whole 3.0-kb human β globin gene insert. Fewer than 10% of the K562 clones lacked the transferred human β globin gene. When the DNAs were cut with *Sst*I, an enzyme that creates many distinctive fragments of the viral DNA, and probed with adenovirus 5 DNA all the expected fragments were detected showing integration of the entire adenoviral vector genome (data not shown). These results agree with previous analysis that showed integration of the entire vector genome in 1−3 copies in rodent fibroblasts and K562 cells (Van Doren *et al.*, 1984; Karlsson *et al.*, 1985).

S1 nuclease analysis was used to search for β globin gene transcripts from the transferred β globin gene in human fibroblasts and K562 cells. The endogenous β globin gene is not expressed in these cells and neither is the transferred β globin gene (Figure 4). Five K562 clones transformed with Ad-hβ/neo have been analysed for β globin gene expression and all were negative.

The hybrid globin gene for Ad-γ−β/neo is expresed in K562 cells

The Ad-γ−β/neo virus was purified on a cesium equilibrium gradient to obtain a higher titer virus. The purified virions were used to transform VA2 human fibroblasts and K562 cells and G418-resistant mixed populations (VA2) and individual clones

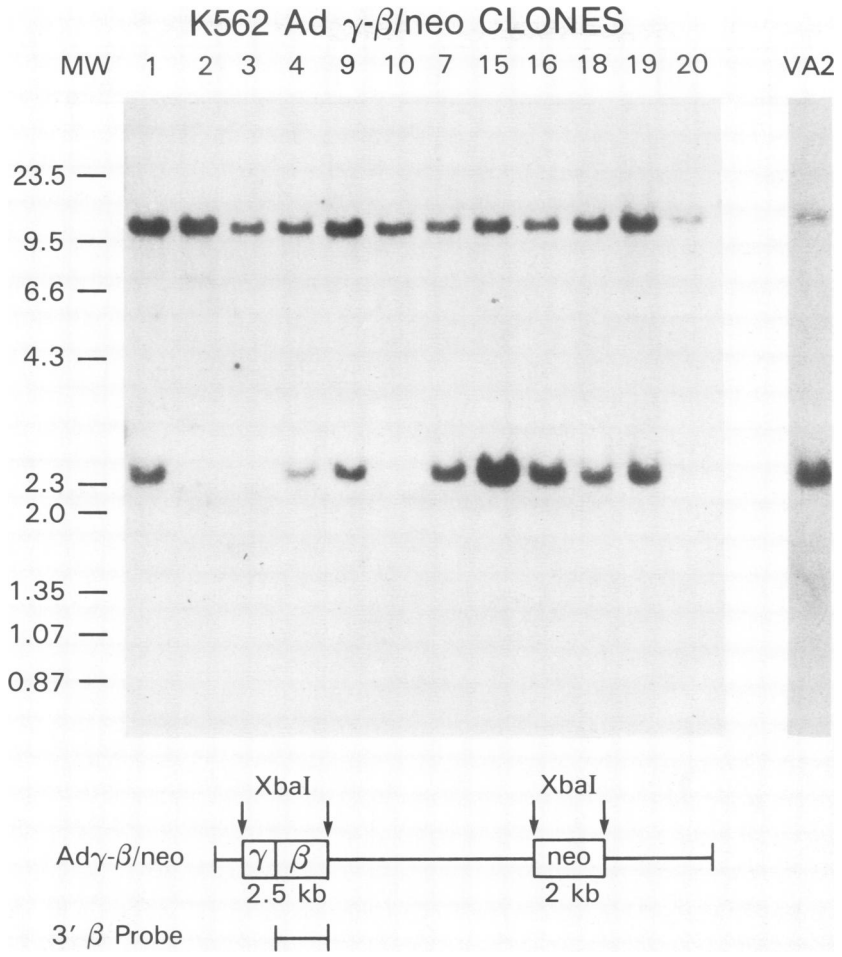

Fig. 5. Southern blot analysis showing the presence of the 2.5-kb γ−β hybrid globin gene in VA 2 cells and many K562 clones transformed with the Ad-γ−β/neo virus. The K562 clones no. 2, 3 and 10 did not contain the γ−β gene. The DNA samples were cut with *Xba*I and probed with the 3′ part of the human β gene. The 2.5-kb band is seen in clone 20 although the signal is weak here due to loss of DNA during processing of the samples. The endogenous β globin gene is also seen as a high mol. wt band.

(K562) were isolated. DNA from these cells was analysed by Southern blot analysis and found to contain the entire 2.5-kb γ−β gene transcription unit with the exception of three G418-resistant K562 clones (Figure 5). Nine of 12 K562 clones (75%) analysed contained the γ−β gene. When these samples are probed with adenovirus 5 DNA the fragments seen indicate integration of the entire viral genome when both the globin gene in the E1 region and the *neo*^{R} gene in the E3 region are present in the transformed cells (data not shown).

Since the endogenous human γ globin gene is expressed in K562 and shows low level induction with hemin we looked for expression and inducibility of the transferred γ−β hybrid gene in eight of the K562 clones containing the γ−β gene and one clone that did not (Figure 6). Clone no. 10 does not contain the γ−β gene and is therefore negative in the expression analysis. All eight clones containing the γ−β gene express it at the RNA level as is shown by the 212-bp protected fragment from exon 3 of the human β globin gene in the S1 nuclease analysis shown in Figure 6. Four of the clones (4, 9, 16 and 19) do not increase their γ−β mRNA upon induction with hemin, and three of them even had reduced levels in the induced samples. All these clones exhibit relatively high level expression in the uninduced state. The clones that exhibit a lower level of expression in the uninduced state (1, 7, 18, 20) are all shown to increase their γ−β mRNA level 2-fold upon hemin induction as determined by scanning densitometry. It is therefore difficult to state that we have achieved inducible expression of the transferred γ−β globin gene in K562 cells.

We have compared the expression level of the endogenous γ globin gene in K562 cells with the expression level of the transferred γ−β globin gene in both the K562 clones and the VA 2 cells transformed with Ad-γ−β/neo. Figure 7 shows the S1 nuclease analysis of the clones using a probe for exon 1 and 2 of the γ gene that detects both the endogenous γ gene and the transferred γ−β gene (there is complete sequence homology between exon 2 of the γ gene and the γ−β hybrid gene). Also shown is a probe that detects exon 3 of the β gene and therefore detects only the expression of the transferred gene. These data show that 2 μg of RNA probed with the γ probe give a considerably stronger signal than 20 μg of RNA probed with exon 3 of the β gene indicating that the steady-state level of the mRNA from the transferred γ−β gene is only present at a 1−3% level of the endogenous γ gene mRNA. Figure 7 also shows that the expression level of γ−β in the VA 2 fibroblasts is barely detectable.

Ad-γ−β/neo transformed cells show independent regulation of the γ−β globin and neo^R *genes*

We have also analysed the level of mRNA transcribed from the *neo*^{R} gene in the VA 2 fibroblasts and all the K562 clones transformed with Ad-γ−β/neo. There is no direct relationship

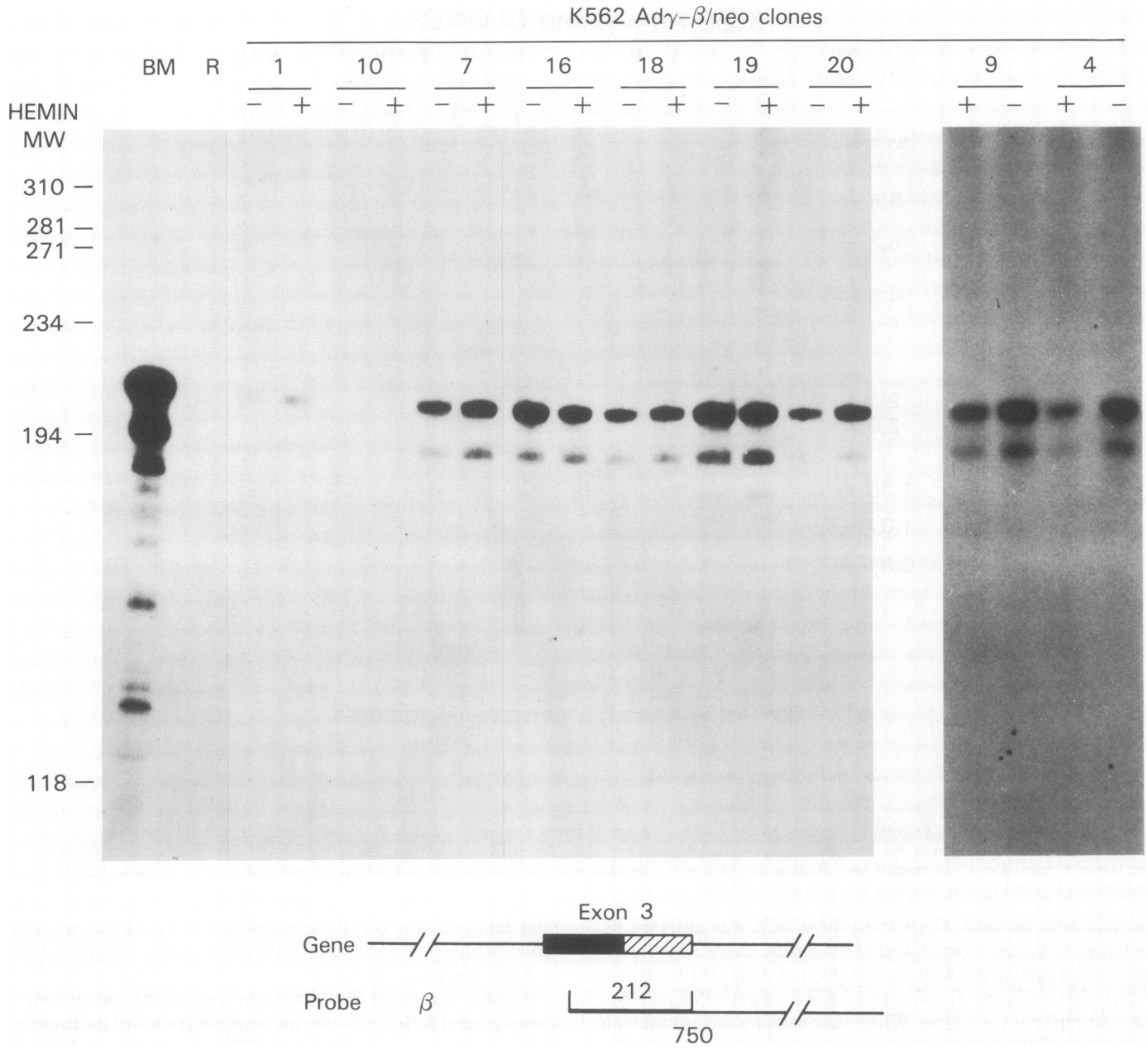

Fig. 6. S1 nuclease analysis of RNA samples from induced (+) or uninduced (−) K562 cells that have been transformed with the Ad-γ−β/neo virus. BM = bone marrow, R = tRNA, MW = mol. wt markers. The β globin probe used is shown below. It should give a 212-nucleotide-long protected fragment. The 212-nucleotide fragment is seen in all samples except clone 10 which does not contain the γ−β gene (Figure 5). The autoradiographs were exposed for 18 h.

between the level of *neo*^{R} gene expression and the level of γ−β expression in these clones. In some cases the *neo*^{R} mRNA is increased upon induction and in other cases not, but there is no relationship between an increase in *neo*^{R} mRNA and γ−β mRNA upon induction. For example clone 4 shows reduced γ−β mRNA upon induction whereas *neo*^{R} is increased. Figure 8 shows an example of the *neo*^{R} mRNA level as determined by S1 nuclease analysis in the VA 2 fibroblasts transformed with Ad-γ−β/neo and a few of the K562 clones. The fibroblasts showed a high level of *neo*^{R} mRNA but γ−β globin mRNA was barely detectable. In general all the K562 clones showed a relatively high level of *neo*^{R} mRNA in the uninduced state (Figure 8 and data not shown) whereas a considerable variability was in the γ−β globin mRNA expression level in the same clones (Figure 6).

Discussion

Tissue-specific expression of the γ−β hybrid globin gene

We used an adenoviral vector system to introduce two genes, a *neo*^{R} gene and a human globin gene (β or a γ−β hybrid gene) into fibroblasts and K562 human erythroleukemia cells. The *neo*^{R} gene was expressed in both fibroblasts and K562 cells and the level of expression was similar in these cells. This would be expected since the gene is driven by the SV40 early promoter which is known to be very active in many cell types.

In contrast the transformed globin genes (β and γ−β) were not expressed in the fibroblast cell line. In K562 cells the γ−β hybrid globin gene was expressed but not the β globin gene. Furthermore four out of eight clones showed a 2-fold increase in γ−β mRNA upon induction with hemin or slightly lower than the mean induction increase of the mRNA from the endogenous

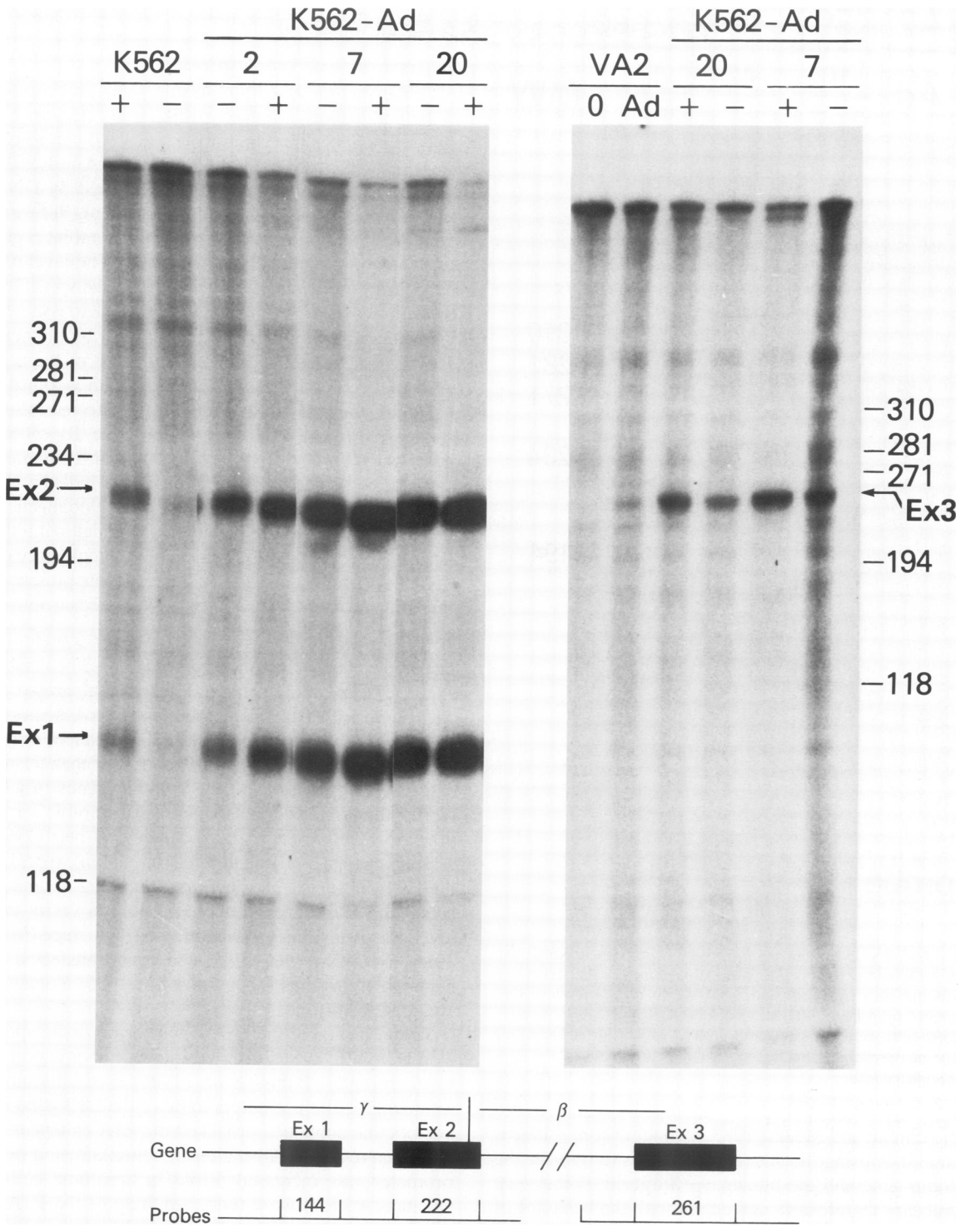

Fig. 7. Comparison of the levels of mRNA expression from the endogenous γ gene and the transferred γ−β gene in Ad-γ−β/neo transformed K562 clones. On the left untransformed K562 cells and Ad-γ−β/neo clones no. 2, 7 and 20 are analysed with a probe detecting RNA corresponding to exon 1 and exon 2 from the endogenous γ gene in K562 and clone 2 (lacks the transferred γ−β gene) and the endogenous γ gene plus the transferred γ−β in clones 7 and 20. + indicates induction with hemin but − indicates no induction. On the right a detection of the 261 nucleotide β globin exon 3 fragment is shown, detecting only mRNA from the transcription of the transferred γ−β globin gene. Two clones 7 and 20 containing the γ−β gene and VA 2 cells transformed (Ad) or not transformed (0) with Ad-γ−β/neo are analysed.

γ globin gene (Charnay and Maniatis, 1983; Dean *et al.*, 1983). Our induction data are comparable with results obtained with a γ−β hybrid gene transfected into K562 cells where some of the clones showed inducibility (Kioussis *et al.*, 1985). The low level increase in γ−β mRNA upon induction in 50% of the clones is hardly very meaningful.

The fetal γ globin gene is expressed in K562 cells but the adult β globin gene is not (Dean *et al.*, 1983). A cloned β globin gene from the K562 cell line exhibited normal function after introduction into COS cells (Fordis *et al.*, 1984) but the chromatin structure of the β globin genes in K562 cells seems to have an inactive conformation as indicated by the absence of DNase I hypersen-

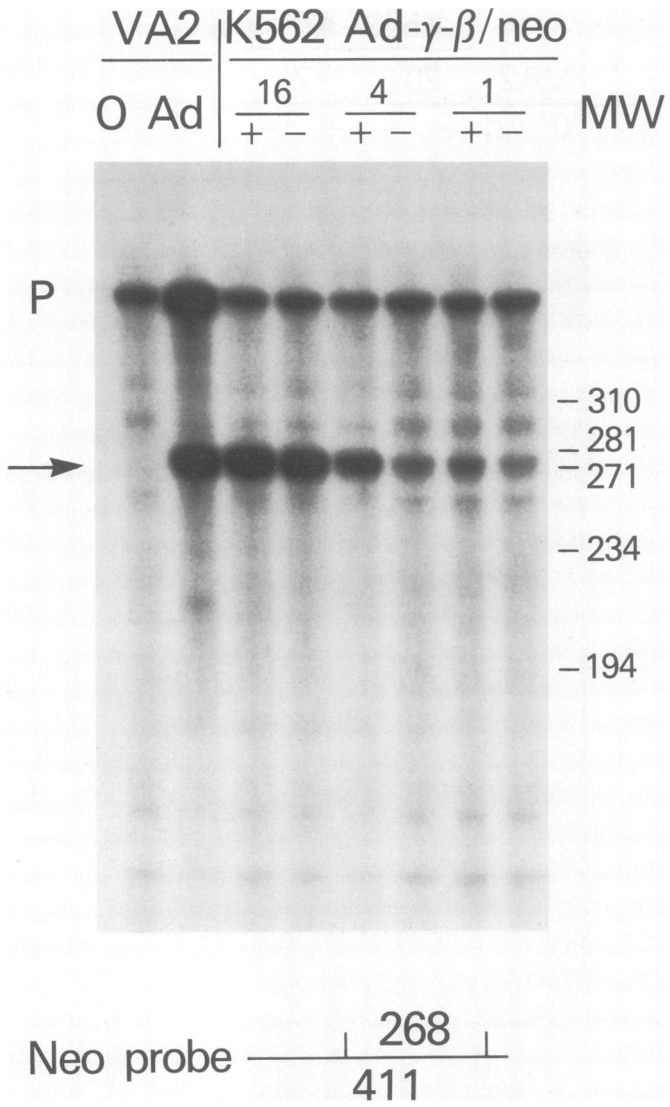

Fig. 8. An S1 nuclease analysis showing *neo*^{R} gene expression in K562 clones and VA 2 fibroblasts transformed with Ad-γ−β/neo. VA 2 cells transformed with the virus (Ad) and control VA 2 cells not transformed (0) are shown. Samples from three different clones (1, 4, 16) of Ad-γ−β/neo transformed K562 cells were analysed, both induced (+) or not induced (−) with hemin. The neo probe depicted below should yield a protected fragment of 268 nucleotides.

sitive sites (Lachman and Mears, 1983; Groudine *et al.*, 1983). Our expression data using the adenoviral vectors to transfer the human β globin gene and the human γ−β hybrid globin gene into K562 cells are therefore in agreement with the behavior of the endogenous globin genes in these cells and demonstrate proper tissue-specific regulation of the transferred γ−β globin gene in these cells. Furthermore the SV40-*neo*^{R} gene and the γ−β globin gene are independently regulated in K562 cells and fibroblasts.

Adenoviral vectors have also been used to obtain tissue-specific expression in other biological systems. Friedman *et al.* (1986) have recently shown that an adenovirus vector system lacking the E1A enhancer can be used to obtain tissue-specific expression of genes in hepatoma and myeloma cells. The expression analysis was done 6−24 h after the viral infection. Also Reuther *et al.* (1986) have shown that a hybrid gene consisting of the E1B promoter and a murine immunoglobulin μ gene can create μ mRNA which undergoes cell-type specific post-transcriptional regulation in myeloma cells.

The level of γ−β gene expression

It is clear that the γ−β globin mRNA is low compared to that of the endgoenous γ gene in K562 cells (Figure 8). Repeated analysis indicates that the transferred γ−β gene expresses only 1−3% of the endogenous γ globin mRNA in K562 cells. This is considerably lower than can be obtained by DNA-mediated gene transfer techniques. Kioussis *et al.* (1985) found tissue-specific expression of a human γ globin−rabbit β globin hybrid gene (junctional *Bam*HI site in exon 2). The level of expression of the transferred human−rabbit hybrid gene was at least 10 times lower than that of the endogenous gene (Kioussis *et al.*, 1985). A human γ−β globin gene containing a γ globin gene promoter (1.6 kb of upstream sequences) and most of the γ globin gene down to the *Eco*RI site in exon III where it is joined to a human β globin gene was constructed and transfected into K562 cells. The γ−β mRNA level was found to be 10−50% of the endogenous γ gene mRNA level (Donovan-Peluso *et al.*, 1985; A.Bank, personal communication). This indicates that a higher level of expression can be expected from globin genes transfected into K562 cells than can be obtained by adenoviral transfer. One can also argue that there is some inhibitory influence of the human β globin intron II sequences in our vector not present in the hybrid genes mentioned above that were transfected into K562 cells.

Possible influence of E1A enhancer on expression

The K562 clones transformed with Ad-γ−β/neo that we analysed contained the entire adenoviral genome integrated. As a consequence the E1A enhancer must always have been just upstream from the globin gene since the E1A enhancer is present in all our vectors. This proximity of the E1A enhancer may possibly explain expression of the γ−β globin genes in all clones that contain them. However, there is considerable variation in expression levels from one K562 Ad-γ−β/neo clone to another arguing against a profound effect on globin expression levels by the E1A enhancer. It is therefore tempting to conclude that the actual site of integration of the adenoviral vector influences the level of expression as is seen in transgenic mice (Magram *et al.*, 1985; Townes *et al.*, 1985) and cells transfected with globin-containing plasmids (Kioussis *et al.*, 1985; Young *et al.*, 1984; Anagnou *et al.*, 1986).

The influence of the E1A enhancer on tissue-specific expression of hybrid genes inserted into adenoviral vectors has been tested recently. Babiss *et al.* (1986) used albumin, globin and immunoglobulin promoters to drive the E1B gene in adenoviral vectors that contained or lacked the E1A enhancer. The mRNA level (harvested 6−24 h after infection) created by the albumin promoter was increased 5-fold in hepatoma cells when E1A enhancer vectors were used. Addition of the E1A protein further increased the mRNA level to 100-fold that of the basic level (no E1A enhancer, no E1A protein). The tissue-specific expression in hepatoma cells can be increased by the presence of E1A enhancer and E1A protein but the E1A enhancer/protein combination cannot turn expression on if the albumin promoter is replaced by a globin promoter.

The E1A protein can activate expression of endogenous cellular genes (Stein and Ziff, 1984; Rosenthal *et al.*, 1985) and transferred viral genes (Curtois and Berk, 1984). The E1A protein can also have a negative regulatory effect by repressing enhancer-induced stimulation of transcription (Borelli *et al.*, 1984; Velcich and Ziff, 1985). It is conceivable that our low-level expression of the γ−β globin reflects repression by a *trans*-acting factor

in the K562 cells of the E1A enhancer upstream from the globin gene in our vectors but there is no evidence to date that cellular encoded *trans*-acting factors can repress viral enhancers that are integrated into cellular chromosomes.

Adenoviral vectors compared with other viral vectors

The inserted genes in the adenoviral vectors studied here remain stable during repeated passage through 293 cells. Both human globin genes were functional in 293 cells and their mRNAs were found to be normally initiated, spliced and terminated.

We could easily transfer a globin gene and a neo^R gene belonging to the same adenoviral vector into all the cells tested here, showing that the non-selectable globin gene located more than 20 kb from the selectable neo^R gene is transferred along with the neo^R gene in most cases (75–90%). Since a considerable fraction of the adenoviral genome can be replaced with inserted foreign sequences (7 kb in our vector system) and since the transformation frequency is easily detectable in a variety of cell lines (Van Doren *et al.*, 1984; Karlsson *et al.*, 1985; Ruether *et al.*, 1986; Babiss *et al.*, 1986; Friedman *et al.*, 1986), it is clear that this vector system can be used to study tissue-specific regulation of genes in a variety of cell lines.

However, we have not found this vector system useful for stable transfer of genes into stem and progenitor cells of bone marrow (unpublished data) as can be done using retroviral vectors (Williams *et al.*, 1984; Hock and Miller, 1986; Gruber *et al.*, 1985). This disadvantage, a relatively low specific transformation frequency, is in fact the major disadvantage adenoviral vectors have when compared to retroviral vectors.

In summary, adenoviral vectors have proven to be useful vehicles for gene transfer in a variety of cell lines propagated in tissue culture. The transferred genes exhibit tissue-specific regulation and when two genes are transferred simultaneously they are independently regulated. They compare very well to other DNA viruses as vectors for gene transfer but have a lower transformation frequency than retroviruses and are therefore probably of limited use in transferring genes into stem cells of bone marrow and embryonic cells.

Materials and methods

Cells

VA 2 human fibroblasts supplied by Dr V.Gopal, CHO DG 21 hamster fibroblasts obtained from Dr L.Chasin and 293 cells were all grown in Iscove's modified Eagle's medium (IMEM) containing 10% (v/v) fetal calf serum. After viral infection the 293 cells were grown in IMEM and 2% gamma globulin-free calf serum. K562 cells were grown in RPMI-1640 medium containing 10% fetal calf serum. The fibroblast cells were selected in 1 g/l G418 (dry weight) and the K562 cells in 2 g/l G418.

Construction of recombinant viruses

Standard techniques were used to construct recombinant plasmids (Karlsson *et al.*, 1985). The recombinant adenoviruses were made by *in vitro* ligation or *in vivo* recombination and propagated in 293 cells. The details of the construction procedures are described elsewhere (Gluzman *et al.*, 1982; Van Doren *et al.*, 1984; K.Van Doren and Y.Gluzman, in preparation). Briefly the construction was accomplished in one of two ways. *In vitro* ligation of the inserted gene with dephosphorylated viral arms was performed and the ligation mixture transfected into 293 cells. *In vivo* recombination was also used. This involves construction of a plasmid containing adenoviral sequences in the middle of which the inserted gene is placed. This plasmid is linearized and co-transfected into 293 cells with a subgenomic adenoviral fragment. In the 293 cells the adenoviral fragment and the viral sequences in the plasmid undergo homologous recombination creating the final viral construct. 293 cells can propagate these E1-defective viruses since they express the E1 gene products.

Following the transfection plaques are picked and tested for presence of recombinant virus by a DNA dot blot procedure and probed with the inserted gene. The positive viral lysates are then used to reinfect 293 cells, low molecular weight DNA is isolated (Hirt, 1967) and the structure of the recombinant virus is analysed and the orientation of the insert determined.

DNA and RNA analysis

DNA was prepared by standard methods and analysed by Southern blot analysis. RNA was isolated by the guanidine thiocyanate technique (Chirgwin *et al.*, 1979) and analysed by S1 nuclease mapping (Ley *et al.*, 1982; Anagnou *et al.*, 1986).

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